CELL DIVISION IN A SPECIES OF *ERWINIA*[1, 2]

II. Inhibition of Division by D-Amino Acids

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Cell division in a species of *Erwinia* can be inhibited by certain carbon-energy sources when the cells are grown in nutrient broth (Grula, 1960). Because nutrient broth is an ill-defined complex, it was decided that nutritional relationships and the accumulation or the disappearance of a compound(s) essential to cell division could best be studied using a chemically defined medium.

MATERIALS AND METHODS

Basic techniques and conditions have been reported (Grula, 1960). All chemicals used in this study were of the highest quality obtainable and, in many instances, the same chemical was obtained and tested from several supply houses.

During the early phase of this investigation, the cells appeared, at times, to lose their ability to elongate unless they were periodically transferred to nutrient agar containing 1 per cent glucose. Therefore, the cells were routinely transferred from nutrient agar to glucose nutrient agar and vice versa every time a transfer was involved (usually every 24 hr during active periods of experimentation and at least monthly during less active periods). Cells grown on nutrient agar (no glucose) were always used as inoculum for studies involving defined media. Lack of consistent elongation was probably due more to lack of knowledge concerning the process than to the medium used, since, at the end of this study, a culture that had been transferred routinely on nutrient agar 52 times during a 3-month interval formed long cells when transferred back to nutrient agar glucose medium.

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[2] Portions of this study were presented at the 59th General Meeting of the Society of American Bacteriologists, St. Louis, Missouri, May 10 to 15, 1959.

RESULTS

Formulation of a chemically defined medium for growth of Erwinia and other phytopathogens has been reported (Starr, 1946; Starr and Mandel, 1950). The medium has the following composition per 100 ml: glucose, 0.5 g (autoclaved separately); NH_4Cl, 0.1 g; KH_2PO_4, 0.2 g; $MgSO_4 \cdot 7HOH$, 0.02 g (solution I); and the following as trace mineral salts, H_3BO_3, 0.5 μg; $CaCO_3$, 10.0 μg; $CuSO_4 \cdot 5HOH$, 1.0 μg; $FeSO_4(NH_4)_2SO_4 \cdot 6HOH$, 10.0 or 50.0 μg (we used the 50 μg level); KI, 1.0 μg; $MnSO_4 \cdot HOH$. 2.0 μg; MoO_3, 1.0 μg; and $ZnSO_4 \cdot 7HOH$, 5.0 μg (solution II). The pH was adjusted to 6.8 with NaOH.

When washed cells of Erwinia were added to this medium and incubated for 1 week at 25 or 30 C on a rotary shaker, growth did not occur. Addition of either Casamino acids (acid or enzymatically hydrolyzed) or a mixture of amino acids (Henderson and Snell, 1948), permitted excellent growth in less than 24 hr. Therefore, individual amino acid solutions were prepared and added to the defined medium. Levels of each amino acid tested were as follows: DL-α-alanine, DL-aspartic acid, L-glutamic acid, 1.6 mg/ml medium; L-arginine, DL-isoleucine, DL-methionine, DL-phenylalanine, DL-serine, DL-valine, 0.32 mg/ml medium; glycine, DL-histidine, DL-leucine, and L-proline, 0.16 mg/ml of medium. After 16 hr incubation at 25 C, aspartic acid supported best growth, approximately double the growth in the presence of glutamic acid, valine, and histidine, which were next best. Alanine, arginine, isoleucine, phenylalanine, and leucine supported only slight growth and methionine, serine, glycine, and proline gave no growth. At 40 hr, each amino acid supported growth, although growth in the presence of serine was poor. Therefore, although any of the amino acids tested could support growth, aspartic acid gave the most rapid proliferation of cells. Cell size was also observed at 16 and 40 hr and found to vary

between 3 and 5 μ for all amino acids except DL-serine. Cells grown in the presence of serine, as the sole source of organic nitrogen, were small (1 to 2 μ).

Various combinations of several amino acids (aspartic, glutamic, valine, methionine, and histidine) were added in an attempt to increase cell length in the defined medium. At no time could elongation greater than 5 μ be obtained. The thinking at this stage of the experimentation was: if the correct conditions for inhibition of cell division could be obtained, cells should become 100 μ or greater in length rather than 5 or 10 μ.

Because elongation could not be increased using the various amino acid combinations, individual solutions of B-vitamins were added to the defined medium containing aspartic acid even though it was obvious that B-vitamins were not required for growth. Although none of the vitamins were capable of increasing cell size to any great extent (none greater than 8 μ), it was noted that *p*-aminobenzoic acid (40 to 400 μg/ml) retarded growth (turbidimetric) and cell size was again small (1 to 2 μ). Addition of individual purine and pyrimidine bases (adenine, guanine, xanthine, uracil, and cytosine) to a level of 20 μg/ml also did not enhance elongation when added to the defined medium.

Because certain sugars interfere with cell division (Grula, 1960), various acids or their salts, occurring as intermediates in sugar metabolism, were tested. The sodium salts of formate, acetate, butyrate, pyruvate, potassium fumarate, and succinic, malic, and α-ketoglutaric acids were filter sterilized, the acids adjusted to neutral pH with KOH, and added to the medium in place of glucose to a level of 0.0336 M concentration. Pyruvate was the only organic acid able to replace glucose and cause long cell formation; however, some elongation occurred in the presence of succinic acid. With most of the compounds tested, cell size was intermediate (2 to 4 μ), and at no time were cells longer than those grown in the presence of glucose. Butyrate, acetate, formate, and malic acid were toxic, whereas the other compounds allowed good growth.

Because various amino acid combinations, B-vitamins, purines, and pyrimidines or acid metabolic intermediates would not allow elongation in the defined medium any greater than that obtained using glucose, each constituent of the medium was studied since it was not known if all constituents were needed, and the possibility existed that one of them was preventing the cells from becoming extremely long. Therefore, each compound (glucose, $MgSO_4$, KH_2PO_4, aspartic acid, and NH_4Cl) and the trace mineral solution was individually titrated against all other constituents of the medium in "rule-out" experiments. The following observations were made after 16 hr incubation at 25 C: (1) aspartic acid, KH_2PO_4, and $MgSO_4$ were essential for growth, (2) glucose stimulated, but was not required for growth, (3) the trace mineral solution and NH_4Cl were not needed for growth. Because glucose was involved in elongation, it was retained in most future media manipulations and also, the trace mineral solution was retained since it was felt that the solution would compensate for possible fluctuations in the purity of distilled water. Phosphate was added as an equimolar solution of KH_2PO_4 and K_2HPO_4 to give a starting pH of 6.8 without adjustment. Therefore, the basal medium was modified to contain the following per 100 ml of distilled water: glucose, 300 mg (added aseptically); $MgSO_4 \cdot 7HOH$, 3 mg; K_2HPO_4, 174 mg; KH_2PO_4, 136 mg; aspartic acid, 280 mg (made up separately and adjusted to pH of about 7.0 using NaOH); and the trace mineral salt solution (II) as listed at the beginning of this report. Length of cells grown in this medium in the presence of glucose is 5 to 10 μ, with some cells growing to about 20 μ. In the absence of glucose, cell length is uniform and in the 2 to 4 μ range.

Because glucose caused greater elongation in the modified medium, several compounds were re-checked for ability to enhance elongation, particularly amino acids since NH_4Cl had now been excluded. Data for DL forms of the amino acids (except glycine) are given in table 1. After 16 hr incubation, six amino acids, DL-serine, DL-methionine, DL-phenylalanine, DL-histidine, DL-threonine, and DL-tryptophan, caused formation of extremely long cells. The cells grew to 100 to 300 μ in length and appeared as long filaments some of which had division areas every 10 to perhaps 30 μ. A wide variation in growth response to the different amino acids is also evident as shown in table 1.

Addition of DL-serine at the same concentration to nutrient broth in the absence of glucose gave rise to cells of approximately the same length as seen previously using glucose. Addition

TABLE 1

*Response of Erwinia sp. to various amino acids**

Amino Acid	Concn	OD†	Cell Size in μ
	M		
Aspartic acid (no glucose)	0.0169	0.83	2–4
Aspartic acid	0.0169	1.2	5–10; 10% 10–20
Arginine·HCl	0.0336	1.5	3–5
Threonine	0.0336	0.17	10–150
Glycine	0.0336	1.05	4–5; 20% 5–10
Methionine	0.0168	0.85	5–100
Glutamic acid	0.0336	1.3	3–4
Valine	0.0168	0.38	3–10; 10% 10–20
Leucine	0.0336	0.15	3–5; 10% 5–15
Serine	0.0420	0.11	20–300
Phenylalanine	0.0168	0.76	10–150
Lysine·HCl	0.0336	1.1	2–5; 10% 5–10
Isoleucine	0.0336	0.32	2–4
Histidine	0.0336	0.69	10–150
Proline	0.0336	0.92	3–7
Tryptophan	0.0063	0.27	10–100
Tyrosine	0.0042	1.2	3–5
α-Alanine	0.0336	0.5	2–5

* All tubes contained trace mineral solution, phosphate, magnesium sulfate, aspartic acid and glucose as given in the text except tube 1 where glucose was omitted.

† Optical density read at 540 mμ after 16 hr at 25 C. OD of inoculum was 0.47.

of DL-serine to the defined medium in the absence of glucose also gave rise to long cells although total cell mass was less and cell size was more in the 20 to 100 μ range rather than the large and tangled masses of 100 to 300 μ filaments seen in the presence of glucose. A titration of DL-serine in the presence of glucose is given in table 2. Increasing concentrations of DL-serine (up to 0.0504 M) allows for a progressive increase in cell length. At higher concentrations, growth is, for all practical purposes, completely inhibited. A concentration of 0.0336 M DL-serine was chosen for future studies because fairly good growth as well as long cell formation occur at this level. Results similar to these were also obtained using either DL-methionine or DL-phenylalanine.

The D and L isomers of serine, methionine, and phenylalanine were then tested to determine if elongation was due to the L or D form of the amino acid. Long cell development occurred only in the presence of the D isomer with all three compounds (0.0168 M for D-serine, 0.0067 M for D-phenylalanine or D-methionine). Although some "bulb" or sphereoplast formation occurs after about 12 hr incubation (size and number vary), it should be pointed out that at about 24 hr and thereafter, cell size appears to progressively decrease (5 to 30 μ size predominates) and many poorly stained "ghost" filaments remain. The bulb formation is unusual in that one or up to three bulbs can form anywhere along the length of a cell. This observation is similar to that reported by Bachmann and Bonner (1959); however, it has not been demonstrated that these filaments are completely coenocytic. Free spheroplasts appear to be relatively stable in 17 per cent maltose.

TABLE 2

*Titration of DL-serine in the presence of 0.0166 M glucose**

Concn DL-Serine	OD†	Average Size in μ
M		
None	1.05	5–20
0.0084	0.96	10–20
0.0168	0.80	20–100
0.0336	0.40	100–300
0.0504	0.12	100–300
0.0672	0.03	No growth
0.0840	0.02	No growth

* All tubes contained trace mineral solution, aspartic acid, magnesium sulfate and phosphate as given in the text.

† Optical density read at 540 mμ after 15 hr at 25 C on a rotary shaker.

D and L forms of aspartic acid were tested in the presence and absence of either glucose, DL-serine, or both to determine if either isomer had an effect on the division mechanism of the cells. Observation of the cultures and cells at 16 hr showed that total cell mass (optical density) was approximately the same in the presence of either isomer and cell size was the same as though the DL form was present in the medium.

Attempts to reverse long cell formation. Studies were initiated to determine how to reverse elongation in order to obtain information concerning involvement of specific metabolites in the division process.

Although critical studies have not been done, it has been observed that cells from the top of liquid cultures containing D-serine not aerated during growth are typically long (100 to 300 μ), whereas cells taken from the bottom of unshaken

TABLE 3

*Ability of various compounds to reverse filament formation in the presence of 0.0166 M glucose and 0.0335 M DL-serine**

Compound	Concn in Medium	Cell Size in μ at 16 hr
	M	
NH_4Cl	0.0747	3–4
$(NH_4)_2SO_4$	0.0747	3–4
NH_4NO_3	0.0747	3–12
p-Aminobenzoic acid	0.0143	3–4†
D-α-Alanine	0.0336	3–4
L-α-Alanine	0.0336	3–4
D-Aspartic acid	0.0105	100–300
L-Aspartic acid	0.0105	100–300
Adenine	0.00074	25–300†
Guanine	0.00132	15–50
Thymine	0.00396	15–150
Cytosine	0.0045	15–150
Hypoxanthine	0.00366	10–100
Uracil	0.00446	15–150
Adenine + guanine	0.00074 + 0.00132	10–50
Cytosine + uracil	0.0045 + 0.00446	15–150
L(+)-Glutamine	0.024	10–50
L-Proline	0.0139	100–300
L-Arginine	0.011	100–300
Glycine	0.021	100–300
L-Valine	0.0486	5–50
L-Isoleucine	0.0336	5–50
DL-Norleucine	0.0134	100–300
β-Alanine	0.0336	100–200

* All tubes contained trace mineral solution, aspartic acid, magnesium sulfate, and phosphate as given in the text.

† Toxic.

tubes are shorter. This type of observation will merit further study because the effect is quite pronounced. Reducing conditions and particularly—SH groups and division have been linked together by several investigators (Mazia, 1954; Stern, 1956; Nickerson and Falcone, 1956; Hase et al., 1959).

Several types of compounds were studied to determine their effect on reversal. Data are tabulated in table 3. Varying amounts of reversal occur with several different compounds thus making analysis very difficult. Reversal by inorganic ammonium salts indicates that nitrogen metabolism is disturbed by D-serine. The disturbance, perhaps, is not singularly unique since alanine, *p*-aminobenzoic acid, adenine and guanine, glutamine, valine, or isoleucine permit varying amounts of reversal. The data do not allow for any definitive conclusions since those compounds best able to reverse elongation (inorganic salts or alanine) have functions which can be general, particularly when transamination reactions are considered. The mechanism of the formation of long cells by D-serine is not a result of the antagonism of L-serine since L-serine at 3 to 6 times the molar concentration of D-serine (studied at 0.0252 M concentration) could not cause reversal. Apparently, the organism must possess both an alanine and an aspartic acid racemase since either isomer allows equal cellular response.

Studies were also done to determine if compounds related to serine or methionine (structurally and metabolically) could cause cell elongation. These data are presented in table 4. Although methionine is involved in 1-carbon transfer reactions and the D isomer causes elongation in this system, other compounds involved in 1-carbon metabolism such as choline or betaine will not enhance elongation. Also, serine, if it is really involved as one of the compounds formed from the dissimilation of glucose, must be synthesized from precursors other than glycine or ethanolamine since these compounds do not elicit the phenomenon. The inability of substituted serine or methionine compounds (serine ethyl ester, hydrogen substitution on the carboxyl group; *O*-methyl serine, hydrogen substitution on the hydroxymethyl group; glycylserine, hydrogen substitution on the amino group; *O*-phosphoserine, hydrogen substitution on the hydroxymethyl group; acetylmethionine, hydrogen substitution on the amino group) to cause elongation equivalent to serine or methionine makes it appear that serine, for example, acts directly to produce long cells, since substitution in the molecule in either of 3 positions can erase its ability to cause production of long filaments. Data presented in table 5, wherein it appears that DL-α-alanine reverses competitively the serine elongation, make a specific requirement for unsubstituted serine more probable.

Although it is obvious that increasing concentrations of DL-serine markedly inhibit growth, it is apparent that, depending on relative concentrations, alanine can either inhibit or stimulate growth. Inhibition by alanine is most apparent at the lowest serine concentrations and

TABLE 4

Ability of compounds related to serine or methionine to cause cell elongation in the presence of 0.0166 M glucose

Compound*	Concn	OD	Average Size in μ
	M		
Glucose	0.0166	1.05	5–10; 10% to 20
DL-Serine	0.0336	0.41	100–300
DL-Serine ethyl ester	0.0336	1.0	3–5
DL-*O*-methyl serine	0.0336	0.94	5–40
Glycyl-DL-serine	0.0336	1.1	3–6
DL-*O*-Phosphoserine	0.0336	1.1	5–10
N-Acetyl-DL-methionine	0.0336	1.1	3–6
DL-Norleucine	0.028	0.18	5–15
DL-Homoserine	0.0336	0.02	2–4
Sarcosine + isonicotinic acid hydrazide	0.0355 each	No growth	
Choline	0.0066	1.6	2–5
Ethanolamine	0.0336	1.3	2–5
Betaine	0.0336	1.4	2–3

* Sterilized by filtration through sintered glass and added aseptically. All tubes contained trace mineral solution, aspartic acid, magnesium sulfate, and phosphate as given in the text.

alanine inhibition also occurs in the absence of serine. Because of this double inhibition, it is difficult to determine if the effect of alanine on growth in the presence of serine is competitive or noncompetitive. A better appraisal can be made by observing cell size in the presence of varying levels of alanine. The reduction in size is marked and alanine exerts a competitive effect at all levels regardless of the concentration of serine. Further calculation of the data shows that the alanine to serine ratio (molar basis) should be in the order of at least 0.4 to 1 to reduce cell size to an average of about 4.5 μ which can be considered almost complete reversal.

Comparative growth curves showing alanine reversal of serine inhibition are shown in figure 1. Actually, during the first 12 to 14 hr, growth is greater in the absence of alanine. It is to be emphasized that these curves are based on optical density measurements and cannot therefore be related to cell division. Growth curves based on colony counts are difficult to make using long cells, since the cells undergo a certain amount of lysis in the growth medium after about 12 hr. Also, plating diluents used thus far (physiological saline or 0.1 per cent peptone water) permit too much lysis of long cells for accurate counting. Figures 2 to 9 compare cells at different times during their growth and also show swollen

TABLE 5

*Reversal of the DL-serine effect at 3 different levels using varying amounts of DL-α-alanine in the presence of 0.0166 M glucose**

Concn DL-Serine	Concn DL-α-Alanine	OD†	Cell Size in μ
M	M		
0.0168	—	0.73	20–200
0.0168	0.00656	0.88	4–5; 20% 4–10
0.0168	0.0164	0.61	4–5
0.0168	0.0328	0.57	4.0
0.0168	0.0656	0.44	3–4
0.0336	—	0.26	30–300
0.0336	0.00656	0.51	5.0; 20% 10–30
0.0336	0.0164	0.44	2–6; 10% 10–20
0.0336	0.0328	0.39	3.0
0.0336	0.0656	0.30	2–3
0.0672	—	0.013	30–300
0.0672	0.00656	0.17	50% 5–10; 50% 20–30
0.0672	0.0164	0.20	4–7; 40% 10–20
0.0672	0.0328	0.15	3–5; 20% 5–20
0.0672	0.0656	0.09	3–5
—	0.0328	0.29	3–4
—	—	0.90	6–10; some to 30

* All tubes contained trace mineral solution, aspartic acid, magnesium sulfate and phosphate as given in the text.

† Optical density read at 15 hr; inoculum 0.33 OD at 540 mμ.

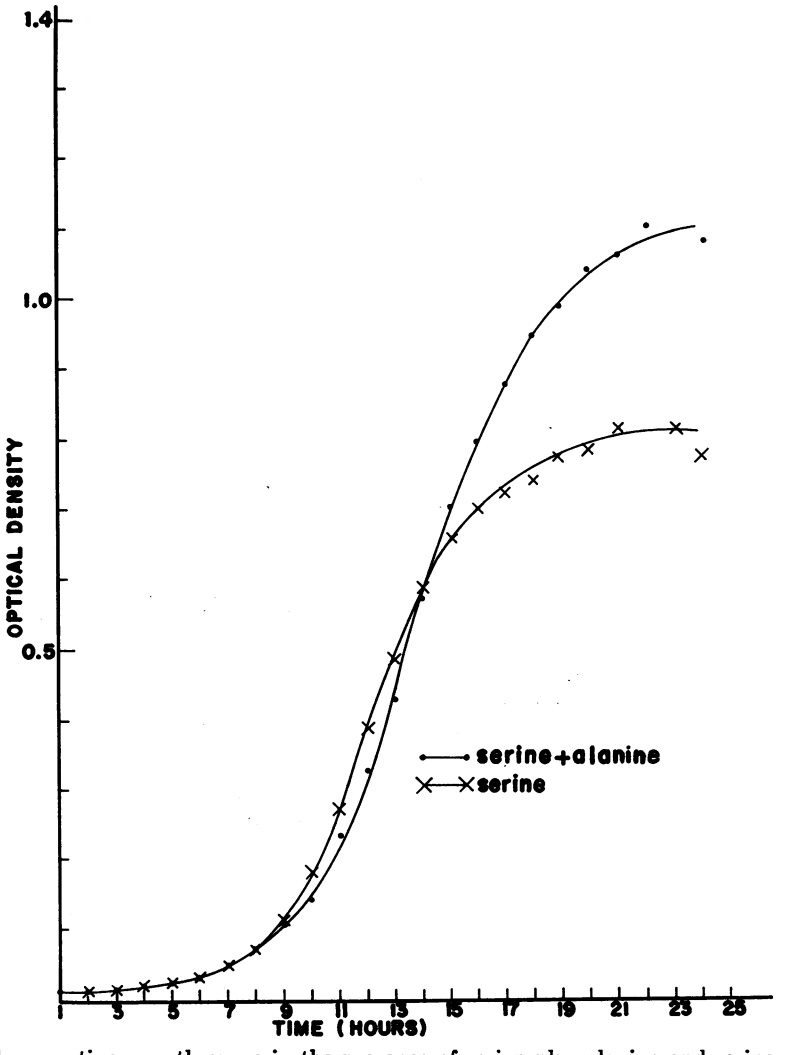

Figure 1. Comparative growth curve in the presence of serine plus alanine and serine alone. (Concentration of serine, 0.0336 M; alanine, 0.0336 M. Both series contained trace mineral solution, aspartic acid, magnesium sulfate, phosphate, and glucose as given in the text.)

cell forms and ghost forms of rods and what we have called spheroplasts. Table 6 is to be compared with figure 1 and relates cell size to optical density measurements. A definite size difference can be observed after 6 hr growth. Bulb formation begins at about 11 hr in the absence of alanine and the greatest number is present at 16 hr. At 19 hr, lysis of the long cells in microscopic preparations is apparent.

DISCUSSION

Results obtained by other investigators who have studied the effects and possible interrelationships of amino acids, particularly the D form, on cells and enzyme systems are varied and point to the complex nature of the over-all problem with regard to growth inhibition, cell division, or spheroplast formation.

Kobayashi, Fling, and Fox (1945) reported that growth of *Escherichia coli* and *Staphylococcus aureus* was inhibited in the presence of D-leucine, D-valine, D-alanine, or glycine. The glycine inhibition could be reversed by D- or L-alanine or by pyridoxine. They suggested that D-amino acids might cause inhibition by interference with "proteosynthetic" enzymes. Either inhibition of

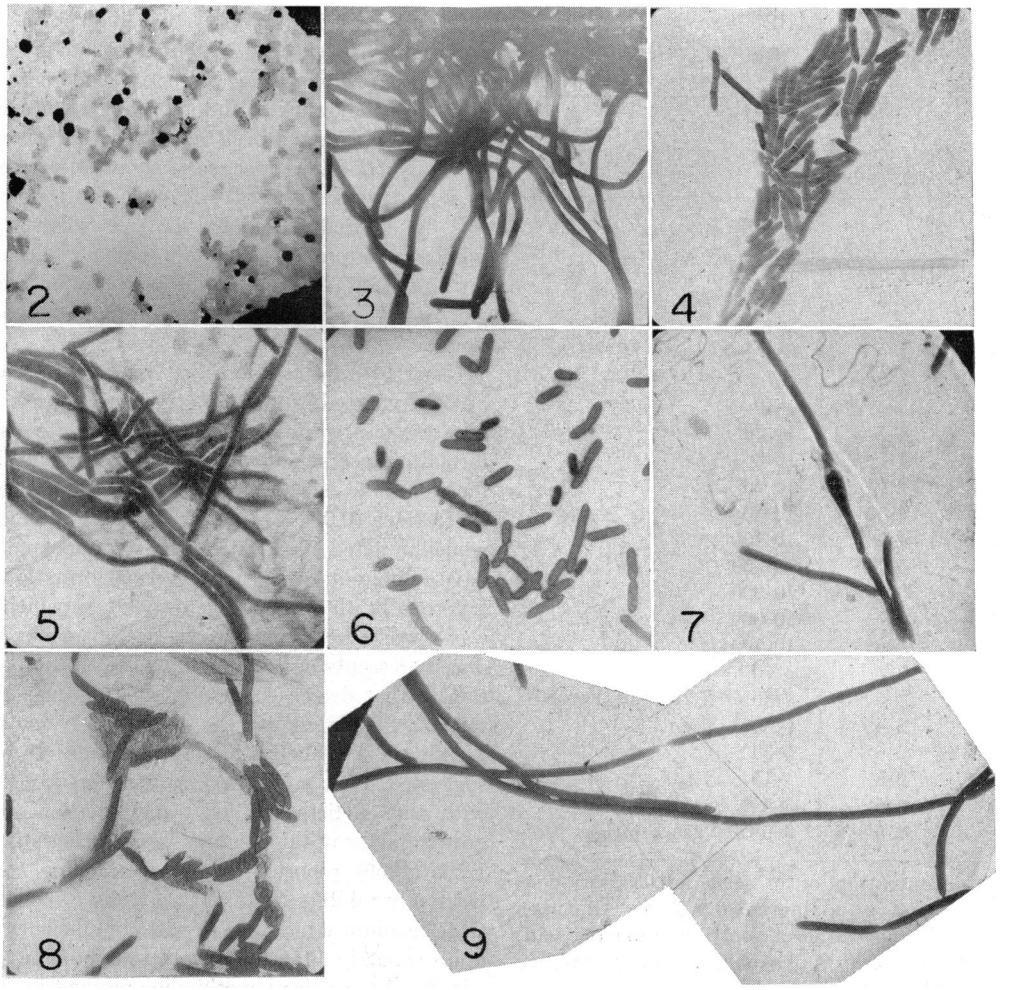

Figure 2. Control showing size of *Erwinia* sp. at zero time. (Dark deposits are probably sodium chloride.) ×1,230.

Figure 3. Tangled mass of long cells after 10 hr growth in the presence of serine. ×1,230.

Figure 4. Short cells after 10 hr growth in the presence of serine and alanine. ×1,230.

Figure 5. Long cells after 35 hr growth in the presence of serine. Note the swollen, distorted forms and the large amount of debris. ×1,230.

Figure 6. Short cells after 35 hr growth in the presence of serine and alanine. ×1,230.

Figure. 7. Long cells after 35 hr growth in the presence of serine. Note the swollen, club-shaped cell. ×1,230.

Figure 8. Long cells after 56 hr growth in the presence of serine. Ghost forms of rods and spheroplasts are present. ×1,230.

Figure 9. Long cells after 24 hr growth in the presence of serine. ×1,230.

Figures 2–9 reduced about 32 per cent for reproduction here.

growth of *Salmonella typhimurium* and *Haemophilus influenzae* or the formation of "round bodies" (spheroplasts?) by DL-methionine, tryptophan, tyrosine, L-phenylalanine, or glycine was reported by Dienes and Zamecnik (1952). The effects did not occur when di- or tripeptides of glycine were substituted for glycine. Yaw and Kakawas (1952) noted that D-methionine, D-valine, or D-leucine inhibited growth of *Brucella abortus* in Albimi broth (nonsynthetic). They suggested that the D-amino acids interfered with hydrolysis of the peptide bond or its synthesis

TABLE 6
*Changes in cell size during growth in the presence of serine and alanine**

Time	Average Size in μ	
	Serine + alanine	Serine alone
hr		
1	1–2	1–2
2	1–3	1–3
3	2–3	2–3
4	2–3.5	2–4
5	3–4	3–5
6	3–5	4–7
7	3–5	5–10
8	3–5	10–30
9	4–7	10–30
10	4–7	10–50
11	4–10	10–80
12	4–10	10–150
13	5–12	10–300
16	3–7	10–300
17	3–6	10–300
18	3–6	10–100
19	3–5	5–70
21	2–5	5–30 (1% greater than 80)
22	2–4	5–30
24	2–6	5–30
29	2–6	4–15
31	2–5	3–6
34	2–5	3–6 (4% greater than 10)

* Concentration of DL-serine, 0.0336 M; concentration of DL-α-alanine, 0.0336 M. All tubes contained trace mineral solution, aspartic acid, magnesium sulfate, phosphate, and glucose as given in the text.

therefore blocking protein synthesis. Rowley (1953) published extensive data on amino acid inhibition and reversal using *E. coli*. Varying degrees of growth inhibition by norleucine were observed in 90 strains, in 75 strains by norvaline, 9 strains by serine, 7 strains by α-aminobutyric acid, 5 strains by aspartic acid, 5 strains by histidine, 3 strains by cystine, 2 strains by lysine, and 1 strain each by leucine or tyrosine. All of the inhibitions could be reversed in nutrient broth; leucine and methionine were the most common reversing agents.

Dawes (1952) made the interesting observation that alanine and glutamic acid deaminases in *E. coli* are aerobic enzymes, whereas the serine and aspartic acid deaminases function best under anaerobic conditions.

Coleman (1959) reported that high levels of D-glutamic acid stopped both division and growth of *Rhodospirillum rubrum*. After extended periods of incubation, growth and morphology became normal. Protein synthesis appeared to continue normally; however, decreased synthesis of nucleic acids was observed. Although the L isomer was preferentially used by the cells when both isomers were present and the L isomer was taken up 2.5 times faster than the D form, the D isomer did not affect cellular incorporation of the L isomer.

Using *Alcaligenes faecalis*, Lark and Lark (1959) reported that all D-amino acids except aspartic acid, glutamic acid, and proline caused formation of crescent forms of the cells. Methionine or valine caused the effect at the lowest concentrations (0.004 M). Although methionine and penicillin acted synergistically, each appeared to have a different target since crescent formation in tryptone broth was possible only when penicillin was used. The D-amino acid and penicillin-induced crescents have a similar cell wall composition in that decreased amounts of alanine, glutamic acid, diaminopimelic acid, and lysine are present in the phenol insoluble portion. It was suggested that D-methionine interfered either with the participation or transformation of a growth factor in the medium. In a further study, Lark (1959) reported that D-methionine competitively inhibits the ability of cells to concentrate medium L-methionine with an efficiency of approximately 14:1. Although D-methionine can specifically displace L-methionine previously concentrated by the cell, it does not interfere with the incorporation of endogenous methionine into cell proteins. Other D-amino acids, such as serine or valine, which also cause crescent formation, do not interfere with the entry of L-methionine into the cell although they block the further increase in the rate of methionine incorporation into protein.

Davis and Maas (1949) reported that although D-serine inhibited growth in a strain of *E. coli*, the D isomer had no close relationship to the metabolism of the L isomer. The inhibitory effect could be completely antagonized by glycine or L- or DL-alanine at concentrations 25 to 100 per cent that of D-serine. In a further study, Maas and Davis (1950) observed that D-serine interfered with the conversion of β-alanine to pantothenic acid; however, they also acknowledged that

higher levels of serine affected metabolic processes other than those involving pantothenic acid synthesis. Their strain was not able to convert D-serine to pyruvic acid, could not readily deaminate D-serine, and ethanolamine did not inhibit growth at a level of 200 μg/ml.

Mueller and Miller (1949) reported that D-serine decreased the yield titer of tetanus toxin, and Kavanagh, Tunin, and Wild (1958) report the increased biosynthesis of cephalosporin N (synnematin B) when D-methionine is added to the growth medium. Although exotic substituted amino acids were used, Martel and Berlinguet (1959) report impairment by some on the development of transplantable Novikoff hepatoma tumor cells.

Eisenstadt et al. (1959) reported that D-aspartic acid inhibited the formation of amylase in cells of *Pseudomonas saccharophila* grown in the presence of maltose, sucrose, or cellobiose. The inhibition could be reversed completely when the ratio of L- to D-aspartic acid was 6:1. The inhibition could also be reversed by adenosine but not inosine and appeared to be due to the inhibition of adenosine monophosphate formation which, in turn, affected the synthesis of adenosine triphosphate, amino acid activation, and synthesis of ribonucleic acid.

A direct effect by a D-amino acid on an enzyme has been studied by Murachi and Tashiro (1958). They observed that the D-isomer of lysine inhibited D-amino acid oxidase by competing with the substrate D-amino acid for the apo-oxidase protein.

Normal synthesis of cellular constituents specifically requiring D-amino acids did not receive serious consideration until D-isomers were found in bacterial cell walls. Recently, a D-alanine activating enzyme has been reported by Baddiley and Neuhaus (1959).

The synthesis of vitamin B_6 by mutants of *E. coli* involves serine, glycine, and glycoaldehyde, particularly at 37 C (Morris, 1959). He suggests that interference with B_6 biosynthesis may be only one consequence of the presence of an incomplete metabolic block which also controls the synthesis of serine and glycine.

Andrejew, Gernez-Rieux, and Tacquet (1958) reported that catalase of mycobacteria and beef liver was inhibited by D-cycloserine. Shockman (1959) was able to reverse the effects of cycloserine on cell wall synthesis in *Streptococcus faecalis* with D but not the L form of alanine. He suggested that the competition between cycloserine and D-alanine might take place at the site of incorporation of D-alanine into a wall precursor. Park (1958) suggested that cycloserine might prevent normal incorporation of D-alanine into the cell wall.

Because D- or L-alanine can reverse D-serine in what appears to be a competitive manner in these cells, and because some spheroplast formation occurs in the presence of D-amino acids, it would appear that some inhibition of cell wall synthesis is occurring. It should be pointed out, however, that cell division is inhibited and the cells become quite long hours before spheroplast formation is evident and, also, many filaments never form any spheroplasts. Because cell wall and other analyses have not yet been done, it is too early to say that the effect of D-amino acids in this system is a direct one which will relate only to uniform cell wall synthesis. The relationships between cell wall synthesis and cell division remain obscure. Although the accumulation of uridine-amino sugar-peptides in the presence of penicillin and interference with cell wall synthesis leading to spheroplast formation have been elegantly described (Park and Strominger, 1957; Strominger, Park, and Thompson, 1959; Lederberg, 1957), the relationship of these amino sugar-peptides to cell division remains to be explained. It is well known that penicillin, for example, will also inhibit cell division causing formation of filamentous cells without concomitant formation of spheroplasts. Is it possible that two somewhat "different" cell walls are synthesized by a cell, one being the wall that is laid down only in the division zone, the other being cell wall that is synthesized at other growing areas or a growing point?

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SUMMARY

The D forms of serine, methionine, phenylalanine, threonine, tryptophan, or histidine either alone or in combination with glucose, profoundly inhibit cell division in a species of *Erwinia* growing in a chemically defined medium. Higher levels of the D-amino acid can also completely

inhibit growth. Division inhibition by serine can be reversed almost completely by inorganic ammonium salts, D- or L-alanine, or *p*-aminobenzoic acid and, to a lesser extent, by other compounds such as guanine, L-valine, L-isoleucine, or glutamine. Reversal of division inhibition by alanine appears to be competitive. Substitution in any of 3 positions in the serine molecule negates division inhibition by serine.

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